Annals of Clinical and Analytical Medicine
Original Research

The relationship between vitamin C and oxidative status in periodontitis: A case-control study

Vitamin C and oxidative status in periodontitis

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Abstract

Aim: A co-factor for reactions including collagen hydroxylation, vitamin C prevents oxidative damage to DNA and proteins and may be associated with oxidative stress in periodontitis. This study was designed to test the hypothesis that vitamin C is inversely related to oxidative stress markers in individuals with periodontitis.

Material and Methods: This study was conducted with 25 individuals with periodontitis and 24 individuals without periodontitis. Vitamin C intake of participants was determined using a valid food frequency questionnaire, and plasma ascorbic acid levels of participants were measured using high-performance liquid chromatography. Total antioxidant status and total oxidant status were measured in serum and saliva samples, and the oxidative stress index was calculated. Lucigenin-enhanced chemiluminescence assays determined superoxide radicals in saliva.

Results: Vitamin C intake in patients with periodontitis was found to be lower than in the control group (124.68 mg/d and 176.71 mg/d, p=0.003, respectively). The plasma ascorbic acid levels and total oxidant status (p=0.03,r=-0.42) and superoxide radical levels (p=0.01,r=-0.53) were inversely correlated in patients with periodontitis.

Discussion: Dietary vitamin C intake in patients with periodontitis was lower than in healthy individuals; vitamin C intake may be inversely related to oxidative stress which is the underlying cause of periodontitis.

Keywords

Periodontitis, Antioxidants, Oxidative stress, Vitamin C, Periodontal disease

DOI: 10.4328/ACAM.21693 Received: 2023-03-15 Accepted: 2023-05-05 Published Online: 2023-05-19 Printed: 2023-07-01 Ann Clin Anal Med 2023;14(7):630-634
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This study was approved by the Clinical Research Ethics Committee of Marmara University, Faculty of Medicine (Date: 2017-01-06, No: 09.2017.035)

Introduction

Periodontal diseases which may cause periodontal tissue destruction, alveolar bone loss and infection are microbial and inflammatory conditions [1]. It is accepted that the main etiologic factor of periodontal diseases is the microbial agents in dental plaque. However, the pathogenesis and etiology of this disease are determined in addition by age, gender, low socioeconomic status, obesity, genetics, tobacco and alcohol use, endocrine disorders, neurologic status, local factors related to teeth, nutritional status and oxidative stress [2, 3]. Though many diseases are associated with oxidative stress, it remains unclear whether oxidative stress is the cause or the result of periodontitis. To prevent or treat oxidative stress related to periodontitis, factors that may cause free radical formation such as smoking, alcohol, ultraviolet light and extreme heat should be avoided and consumption of antioxidant nutrients such as selenium, vitamin E and vitamin C should be increased [4].

Vitamin C has antioxidant effects on free radicals and inhibits their negative effects on the body, sweeping them up and preventing oxidative damage to lipids and proteins. Vitamin C is involved in synthesizing intercellular materials such as collagen fibers in connective tissue and the bone-tooth matrix and plays a role in regulating the immune system. Because of these functions, low vitamin C concentration in the serum and/or plasma may contribute to risk factors of periodontitis [5-7].

This study hypothesizes that there are inverse relationships between vitamin C and oxidative stress markers in individuals with periodontitis and evaluates the relationships between vitamin C intake, plasma ascorbic acid levels (PAAL) and parameters related to oxidative stress in individuals with periodontitis.

Material and Methods

This study is a case-control study. The sample size was determined as 24 for both study and control groups ($p = 0.05$, power 80%). Forty-nine individuals (control group n=24, periodontitis group n=25) aged 18 to 65 participated in this study.

All participants were informed about the study and approved the consent form. Ethical approval for this study was obtained from the Marmara University Faculty of Medicine's Clinical Research Ethics Committee (Protocol no: 09.2017.035).

The exclusion criteria included:

- Periodontal treatment within 6 months of the study
- Tooth extraction within 2 weeks of the study
- Any systemic disease
- Vitamin C supplement use within 3 months of the study
- Antibiotic use within 6 months of the study
- Alcohol or tobacco use
- Pregnancy or lactation

Periodontal Evaluation

In all groups, periodontal status was evaluated with plaque index (PI) and gingival index (GI); in addition, clinical attachment level (CAL) and probing pocket depth (PPD) were measured. Cases, where participants showed bleeding on probing (BP), were documented. All periodontal measurements were performed by one individual using the Williams periodontal probe (Hu-Friedy, USA). All clinical measurements were made from 6 points of teeth (mesiobuccal, buccal, distobuccal, distolingual, lingual, and mesiolingual). The mean PI/GI/PPD/CAL/BP of the individuals was obtained by taking the average of these values. PI, plaque index, determines the thickness of the plaque extending along the gingival margin; to see the plaque, the teeth are air-dried and the plaque is not stained. GI, gingival index, evaluates the gingiva; the probe tip is moved around the gingival edge for 30 seconds and inflammation, bleeding and color are evaluated. CAL, clinical attachment level, measures the distance from the cementum boundary to the bottom of the pocket. PPD, probing pocket depth, measures the distance from the free gingival margin to the base of the pocket; the periodontal probe should be held parallel to the long axis of the tooth, advanced by applying a slight force and stopped when it encounters resistance. BP, bleeding on probing, is evaluated according to whether there is bleeding in the regions after measuring the periodontal pocket size.

Determination of Vitamin C Intake

A valid food frequency questionnaire was developed to evaluate the dietary vitamin C intake among healthy Turkish adults. This was used by a dietitian to determine the vitamin C intake of study participants [8]. Average daily consumption, of foods were calculated using the frequency of consumption and the amounts consumed at one time. The Vitamin C content of foods was obtained from the 'United States Department of Agriculture', 'Turkish Food Composition Database' and the 'BeBiS Nutrition Information System 7.1' (Pasifik Company, Turkey). Daily vitamin C intake levels are expressed as mg/day.

Sample Collection and Preparation

Plasma: Blood samples were collected in 10 ml lithium heparin tubes in the morning (8:00-9:00 am) after an overnight fast and before periodontal examination and were centrifuged for 10 minutes at 3000 rpm at 4°C.

Serum: Blood samples collected in dry tubes were centrifuged for 10 minutes at 3000 rpm at 4°C and serum samples were stored at -80 °C until analysis.

Saliva: Individuals spit in sterile specimen containers after fasting for 12 hours and before periodontal examination and samples were stored at -20 °C until analysis.

Determination of Plasma Ascorbic Acid Level (PAAL)

A private laboratory determined ascorbic acid in plasma using high-performance liquid chromatography on the day of the sample collection. PAAL values were reported as mg/L.

Evaluation of Total Antioxidant Status (TAS) and Total Oxidant Status (TOS) in Serum and Saliva

Serum and saliva TAS and TOS levels were measured by the Erel method using TAS/TOS assay kits (Rel Assay Diagnostics). TAS units are mmol Trolox Eqv./L and TOS units are μmol H_2O_2 Eqv./L. Oxidative Stress Index (OSI) units are AU (arbitrary unit) calculated with the formula OSI = [TOS (μmol H_2O_2 Eqv./L) / TAS (μmol Trolox Eqv./L)] × 100 [9, 10].

Analysis of Superoxide Radicals in Saliva

The superoxide radical in saliva samples was measured by chemiluminescence. Lucigenin reacts with superoxide radicals to produce light, which is then measured for 5 min using a luminometer (Berthold EG & G Minilumat LB 9509, Bad Wildbad, Germany). Lucigenin probes (Final 0.2 mmol/L) were added to

saliva samples containing phosphate buffered saline (pH 7.8) and results were expressed as area under the curve of relative light unit (rlu).

Statistical Analysis

Statistical analysis was performed using statistical software packages (IBM SPSS Statistics, version 22.0). The normality of distribution was determined by the Shapiro-Wilk test. Numbers and percentage values were shown for categorical variables, and differences between groups were analyzed. Quantitative data reported included median, mean rank, 25th percentile and 75th percentile values. The Mann-Whitney U test was applied to compare the median values between groups. The Spearman correlation test was used for the correlations between quantitative values. A value of $p<0.05$ was considered significant.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

In this case-control study evaluating the relationship between vitamin C and oxidative status in periodontitis, PAAL, vitamin C intake, serum and saliva TAS, TOS, OSI values, saliva superoxide radical levels and periodontal evaluation results of patients with periodontitis were compared to healthy individuals. The mean age of the control group was 39.08±9.99 (29-62) years, while the mean age of the periodontitis group was 42.52±7.66 years (31-55). There were 13 women (54%) and 11 men (46%) in the control group and 13 women (52%) and 12 men (48%) in the periodontitis group. There were no significant differences between the mean age and gender distribution of the participants in the two groups (p=0.06 and p=0.88, respectively).

Data on the periodontal parameters of individuals were evaluated. In the control group, the number of missing teeth, PI, GI, PPD, CAL and BP were lower than the periodontitis group (statistically significant for all parameters) (Table 1).

The vitamin C intake, PAAL, serum and saliva TAS, TOS, OSI levels and saliva superoxide radical levels of groups were compared. The dietary vitamin C intake was higher in the control group (p=0.003) and the superoxide radical level was higher in the periodontitis group (p=0.001). No statistically significant difference was found between the groups in other parameters (Table 2).

Weak/very weak correlations were found between vitamin C intake and oxidative status and periodontal parameters of participants with periodontitis, but none of these were significant. Significant negative correlations were found between vitamin C intake and superoxide radical level (r=-0.34; p=0.02) and most of the periodontal parameters when all participants were considered together (Table 3).

The Spearman correlation test was used to investigate the relationships between PAAL and parameters related to the oxidative status of individuals and periodontal parameters. In patients with periodontitis, there was a significant negative correlation between PAAL and serum TOS level (r=-0.42; p=0.03) and a negative and moderate correlation between superoxide radical level and PAAL (r=-0.53; p=0.01) (Table 3).

Table 1. Comparison of some clinical periodontal parameters of the groups.

	Control Group (n=24)		Periodontitis group (n=25)		p†
	Median (25-75th percentile)	Mean rank	Median (25-75th percentile)	Mean rank	
The number of missing teeth	0.00 (0.00-1.75)	17.50	4.00 (1.50-7.00)	32.20	0.00*
PI	0.40 (0.25-0.94)	13.32	1.74 (1.22-2.08)	33.40	0.00*
GI	0.16 (0.07-0.44)	11.61	1.66 (1.42-2.08)	34.90	0.00*
PPD (mm)	2.27 (2.12-2.69)	14.93	3.38 (2.91-4.49)	31.98	0.00*
CAL (mm)	0.01 (0.00-0.07)	12.45	1.10 (0.63-1.68)	34.16	0.00*
BP (%)	11.13 (7.92-24.44)	11.55	100 (100-100)	34.96	0.00*

*p<0.05; †Mann-Whitney U Test; PI, plaque index; GI, gingival index; CAL, clinical attachment level; PPD, probing pocket depth; BP, bleeding on probing.

Table 2. Vitamin C intake, plasma ascorbic acid level and oxidative status of participants.

	Control Group (n=24)		Periodontitis group (n=25)		
	Median (25-75th percentile)	Mean rank	Median (25-75th percentile)	Mean rank	p†
TAS (mmol Trolox Eqv/L)					
Saliva	3.73 (2.26-6.06)	27.25	3.08 (1.56-5.18)	22.84	0.28
Serum	3.31 (2.90-3.56)	26.50	3.14 (2.27-3.58)	22.66	0.34
TOS (μmol H2O2 Eqv./L)					
Saliva	4.05 (1.91-9.75)	22.50	6.42 (2.66-15.67)	27.40	0.23
Serum	4.14 (2.93-5.57)	27.59	3.42 (2.41-4.06)	21.66	0.14
OSI (AU)					
Saliva	0.11 (0.04-0.26)	21.42	0.24 (0.07-0.55)	28.44	0.09
Serum	0.12 (0.10-0.16)	23.96	0.13 (0.07-0.16)	25.00	0.79
Superoxide level (rlu/ml)	1397.50 (1140.00-1937.50)	17.73	1920.00 (1666.25-4010.00)	31.27	0.001*
PAAL (mg/l)	8,83 (6,15-11,99)	26.50	7,00 (4,41-11,96)	23.56	0.47
Vitamin C intake (mg/d)	176,71 (137,15–212,40)	31.17	124,68 (102,16-160,66)	19.Ağu	0.003*

*p<0.05; †Mann-Whitney U test; PAAL, plasma ascorbic acid level; TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index.

Table 3. Correlations between PAAL/vitamin C intake and periodontal parameters/oxidative status.

	PAAL						Vitamin C Intake					
	Control (n=24)		Periodontitis group (n=25)		All individuals (n=49)		Control (n=24)		Periodontitis group (n=25)		All individuals (n=49)	
	r	p†	r	p†	r	p†	r	p†	r	p†	r	p†
Periodontal Parameters												
The number of missing teeth	0.17	0.42	0.07	0.65	0.07	0.65	0.33	0.12	-0.09	0.54	-0.09	0.54
CAL	0.08	0.74	-0.04	0.85	-0.10	0.52	0.17	0.45	-0.03	0.88	-0.33	0.02*
GI	0.17	0.44	-0.07	0.72	-0.12	0.41	0.16	0.48	-0.17	0.43	-0.39	0.01*
PI	0.28	0.21	-0.38	0.06	-0.10	0.52	-0.15	0.50	-0.17	0.41	-0.43	0.00*
PPD	-0.10	0.66	-0.02	0.91	-0.03	0.82	0.18	0.43	-0.27	0.20	-0.36	0.01*
BP	0.35	0.12	-0.01	0.98	-0.04	0.82	0.17	0.45	-0.08	0.72	-0.37	0.01*
Oxidative Status												
Serum												
TAS	0.04	0.84	-0.09	0.66	-0.14	0.33	-0.25	0.25	-0.07	0.75	-0.10	0.49
TOS	-0.23	0.28	-0.42	0.03*	0.10	0.49	0.37	0.07	-0.37	0.07	-0.03	0.81
OSI	-0.19	0.40	-0.19	0.36	0.21	0.15	0.21	0.35	-0.13	0.55	-0.01	0.98
Saliva												
TAS	-0.03	0.89	-0.26	0.22	-0.02	0.89	0.03	0.89	-0.32	0.12	-0.07	0.64
TOS	0.24	0.25	0.05	0.81	0.07	0.62	0.03	0.90	0.11	0.62	0.07	0.62
OSI	0.27	0.20	0.22	0.29	-0.06	0.67	0.03	0.88	0.25	0.24	-0.06	0.67
Superoxide level	-0.10	0.66	-0.53	0.01*	-0.27	0.06	0.01	0.96	-0.39	0.06	-0.34	0.02*

*p<0.05; †Spearman Correlation Test; TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index; PI, plaque index; GI, gingival index; CAL, clinical attachment level; PPD, probing pocket depth; BP, bleeding on probing; PAAL, plasma ascorbic acid level.

Discussion

Vitamin C affects oxidative stress, collagen synthesis, and the immune system, and is thought to play a role in the formation and development of periodontitis, which is characterized by oxidative stress, inflammation and periodontal tissue destruction. This study points to the fact that vitamin C intake is lower in individuals with periodontitis than in healthy individuals, and underscores the relationship between vitamin C and serum TOS and saliva superoxide radical levels in individuals with periodontitis.

Our study found dietary vitamin C intakes of the periodontitis group to be significantly lower than the control group; previous studies on this subject differ. Nishida et al. found a relationship between vitamin C intake level and periodontal diseases, while a different study found no relationship between vitamin B1 and vitamin C intake and risk of periodontitis despite finding a positive relationship between vitamin B2 and vitamin A intake and the risk of periodontitis [11, 12]. Our study showed that PAAL of individuals with periodontitis was lower than in healthy individuals (but not significant). Another recent study has shown that vitamin C, vitamin E and reduced GSH levels in plasma, erythrocyte and gingival tissues of individuals with periodontitis are significantly lower than in healthy individuals [13]. Munday et al.'s 2020 study showed that six of 20 people with periodontitis had vitamin C levels below the target range; Isola et al. showed that individuals with periodontitis had lower levels of saliva and serum vitamin C than in the control group [14, 15]. Kuzmanova et al. reported that plasma vitamin C levels significantly lower in individuals with periodontitis compared to controls (8.3 and 11.3 mg/L respectively; p<0.05). But there was no difference between the dietary vitamin C intake of the groups [16].

In periodontal disease, the measurement of antioxidant, oxidant or reactive oxygen metabolites (ROM) levels in saliva is considered more accurate than in serum because many factors affect the systemic oxidative status [4]. To assess the oxidative status of participants in this study, TAS, TOS and OSI were measured in saliva and serum and superoxide radicals were measured in saliva. Baltacıoğlu and colleagues showed that serum and saliva TOS and OSI values of individuals with periodontitis were significantly higher and total antioxidant capacity lower than healthy individuals; they argued that OSI value could be used as a marker for periodontal diseases [17]. In other study, similarly, serum TAS values of individuals with periodontitis were found to be significantly lower than healthy individuals [18]. Some studies evaluating oxidative status in periodontitis measured ROM in serum or saliva. Our study measured the level of ROM in saliva samples by chemiluminescence. Our study found higher superoxide radical levels in the group with periodontitis than in the control group (p=0.001) and found significant correlations between PAAL and superoxide radical levels in individuals with periodontitis. Canakci et al. found that ROM production and activity in the saliva increased and antioxidant activity decreased during periodontal inflammation [19, 20]. Acquier et al. showed significantly higher ROM levels in the saliva of individuals with chronic and aggressive periodontitis than controls and a positive correlation between ROM level and some clinical indexes (CAL and PPD) [21]. In our study, the correlation between PAAL and superoxide radical levels supports the role of vitamin C in the formation mechanism of periodontitis.

A systematic review of the relationship between periodontal disease and vitamin C (2019) reported that low vitamin C intake or low blood ascorbic acid levels increased the severity of the disease and worsened its prognosis [22]. Amaliya et al. showed a negative correlation between plasma vitamin C levels and CAL and reported that vitamin C could affect the severity

of periodontal destruction [23]. In a study of individuals aged 70 years and over, a negative relationship between serum vitamin C levels and CAL was shown [24]. In our study, weak/moderate, negative, and significant correlations were found between vitamin C intake and PI, GI, PPD, CAL, BP.

According to the findings of our study, vitamin C intake is lower in individuals with periodontitis than in healthy individuals, but there is no significant difference between the OSI, TAS, TOS values of the two groups. Despite this, the fact that dietary vitamin C intake is associated with many periodontal parameters suggests that this vitamin may also be effective against periodontitis due to functions (neutrophil extracellular trap formation, inflammation, collagen synthesis etc.) other than antioxidant activity [25].

Methodological limitations of this study included PAAL measurement. Since PAAL was affected more by short-term vitamin C intake than leukocyte ascorbic acid, ascorbic acid measurement was preferred in leukocytes rather than plasma. Also, if there had been more patients in the study, significance might have increased and/or different relationships might have been found.

Conclusion

In conclusion, this study found that individuals with periodontitis have a lower intake of vitamin C and found relationships between plasma vitamin C levels and oxidative status and some periodontal parameters. Well-designed, long-term, longitudinal studies are needed to evaluate the effectiveness of vitamin C supplements and/or dietary interventions to support periodontal treatment.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

Funding: *This work was supported by Marmara University Research Fund (SAG-C-YLP-120417-0149) (https://bapko.marmara.edu.tr/).*

Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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How to cite this article:

Elif Emiroğlu, Tülin Çakır, Meral Yüksel, Fatma Esra Güneş, Ali Çekici, Berna Karakoyun. *The relationship between vitamin C and oxidative status in periodontitis: A case-control study. Ann Clin Anal Med 2023;14(7):630-634*

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